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Functional Interactions Between Unlinked Muscle Genes Within Haploinsufficient Regions of the Drosophila Genome

Theodore Homyk, Jr., and Charles P. Emerson, Jr.

Department of Biology, University of Virginia, Charlottesville, Virginia 22901

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ABSTRACT

Mutations in 13 genes affecting muscle development in Drosophila have been examined in pairwise combinations for evidence of genetic interactions. Heterozygous combinations of mutations in five genes, including the gene coding for myosin heavy chain, result in more severe phenotypes than respective single heterozygous mutant controls. The various mutant interactions include examples showing allele-specific intergenic interactions, gene specific interactions, and allele-specific intragenic complementations, suggesting that some interactions result from the manner in which mutant gene products associate. Interactions that result from alterations in "+" gene copy number were also uncovered, suggesting that normal myofibril development requires that the relative amounts of respective gene products produced be tightly regulated. The importance of the latter parameter is substantiated by the finding that all five interacting loci map to disperse haploinsufficient or haplolethal regions of the genome. The implications of the present findings are discussed in relation to pursuing the phenomena involving genetic interactions to identify new genes encoding interacting myofibrillar proteins, to examine the nature of intermolecular interactions in mutant and normal development and to decipher the quantitative and temporal regulation of a large family of functionally related gene products.

A genetic investigation of complex cellular and developmental functions can reveal mutations that disrupt the function of each essential component involved, regardless of whether the product is abundant or whether its function has been suspected through prior investigations. Extensions of genetic studies, using molecular genetic techniques, furthermore, permit identification and characterization of specific gene products and their temporal and spatial distributions. Inter- and intramolecular relationships also can be revealed through studies of genetic interactions among different mutationally altered gene products.

Studies of mutations affecting subunits of multimeric enzymes reveal two classes of interactions. The first, termed suppression or positive interaction, includes instances in which separate alterations in two mutant polypeptides suppress or complement one another to yield a more functional heteropolymer (Zabin and Villarejo 1975). Another type of suppression involves mutations that allow the product of one gene to substitute for the function of the product of another gene (Miller and Maruyama 1986). In the second category, referred to as negative complementation or negative interaction, mutations in the same or functionally related polypeptides interact to produce a more extreme phenotype than is expressed when either mutation is present singularly. Interactions in this category include intragenic interactions between relatively weak alleles to produce severe phenotypes and intergenic interactions, between recessive mutations in separate genes, to produce "dominant" phenotypes in double heterozygotes. In some instances, interaction of nonlethal mutations has been found to produce lethality. This phenomenon has been termed synthetic lethality (Dobzhansky 1946) and has been interpreted to indicate functional interrelationships between the respective gene products (Lucchesi 1968).

Muscle cells express many functionally important components involved in contraction, and these components are organized in a highly precise manner important for their contractile function (Usherwood 1975; Hoyle 1983). Muscle, therefore, represents an ideal cell type for the study of interrelationships among individual gene products both at the biochemical level, regarding physical interactions, and at a developmental level, concerning the temporal and quantitative regulation of specific gene expression. Mogami and Hotta (1981) showed that myofibrillar components share a tight interdependency in assembly that is revealed by genetic disruption. Their study revealed that muscle-specific mutations affected sets of related myofibrillar proteins, explainable on the basis of hierachial relationships for the incorporation of various myofibrillar components into stable complexes.

The myriad of biochemical interactions among my-

ofibrillar proteins and the pleiotropic effects of single myofibrillar gene mutations on mutant myofibrillar protein patterns, suggested that examination for genetic interactions represents a useful and formidable approach to identify and characterize additional genes coding for interacting muscle proteins. As a test of this hypothesis, we have examined the phenotypes of muscle defective mutations in twelve separate genes when combined pairwise with one another and with each of seven mutations in the myosin heavy chain gene. Evaluation of such combinations reveals that mutations in five genes interact in such a way that double heterozygotes display otherwise recessive phenotypes, including flightlessness, abnormal wing posture and lethality. The pattern of intergenic interactions of muscle genes were nonrandom, showing both intergenic and interallelic specificities. Such specificities could reflect the nature and extent of interaction between the gene products. Furthermore, increasing the dose of the myosin heavy chain (Mhc^{+}) gene, through use of a duplication, was found to reduce or eliminate flight ability in mutants heterozygous for recessive mutations in two genes, demonstrating the additional importance of quantitative relationships between interacting gene products in muscle development. The importance of this latter relationship among interacting muscle gene products is further highlighted by the finding that mutations in all five of the interacting genes map to either haploinsufficient (for flight) or haplolethal regions of the genome.

MATERIALS AND METHODS

Mutant strains: The flightless mutations and their behavioral phenotypes are listed in Tables 2 and 3. Several of the mutations were carried on chromosomes bearing one or more visible mutations and/or maintained over *FM6*, *FM7* or *CyO* balancer chromosomes. None of the visible marker mutations was found to have an effect on flight behavior, wing posture or viability as measured in heterozygotes in the present study. All visible mutations and balancer chromosomes encountered in this study are described in LINDSLEY and GRELL (1968).

Strains $y\ w\ hdp^{2}/FM6$, $y\ hdp^{3}/y\ hdp^{3}$, $cv\ hdp^{4}/FM6$, $y\ cho\ cv\ hdp^{5}/FM6$, *y sdby/y sdby,v* $int^{3}\ f/FM6$, $cv\ sn\ v\ up^{3}/FM6$, $y\ cho\ cv\ gmp^{2}/FM6$, $cv\ sn\ v\ gnd^{2}/FM6$, $y\ cho\ flw^{3}/FM6$, $up^{x}/FM6$ and *ewg/FM6* (DEAK *et al.* 1982), were obtained from B. COTTON and K. V. RAGHAVAN. The strain bearing up^{x} was not labeled and that it contains an up mutation was determined in this study. *Bsh/Bsh* (GRELL 1969), *Ifm(2)1/Ifm(2)1*, *Ifm(2)2/Ifm(2)2*, *Ifm(2)3/CyO*, Mhc^{1}/CyO and *Ifm(3)7/Ifm(3)7* (MOGAMI and HOTTA 1981; MOGAMI *et al.* 1986) were obtained from K. MOGAMI. *Ifm(3)3/Ifm(3)3* was obtained from E. FYRBERG, and *sr/sr* and *rsd/rsd* was obtained from the Drosophila Stock Center at the California Institute of Technology. *Stp* (COLLIER and FINKE 1984) was obtained from G. COLLIER. *Nup* was isolated as an EMS-induced dominant flightless mutation (T. HOMYK, unpublished data). Strain up^{whu} (GRIGLIATTI *et al.* 1973; HOMYK, SZIDONYA and SUZUKI 1980) was obtained from T. GRIGLIATTI. The isolation of the remaining flightless mutations has been reported (HOMYK and SHEPPARD 1977; HOMYK, SZIDONYA and SUZUKI 1980).

Representative mutations in eight complementation groups in the 2C-D region (PERRIMON, ENGSTROM and MAHOWALD 1985) were obtained from N. PERRIMON in strains balanced with *FM7*. These mutations, including *l(1)C204*, csw^{C114}, $l(1)Pgd^{DF958}$, *l(1)DF967*, *l(1)VE651*, usp^{VE653}, *l(1)GF316*, *l(1)EA82* and $l(1)EA82^{HC207}$ were tested for complementation with $fliA^{4}$. As noted, $l(1)EA82^{HC207}$ is an inversion, with breakpoints in 2C3 and 7B1, and fails to complement *l(1)EA82* for lethality. The Triplolethal (Tpl) mutant strains $Ki\ Tpl^{10}/TM3$, $Ki\ Tpl^{17}/TM3$ and $Ki\ Tpl^{38}/TM3$ were obtained from J. LUCCHESI.

Although genetic and phenotypic descriptions of the non-myosin heavy chain muscle defective mutations have been published, the status of several mutations has been revised. Thus, while genetic data suggest that int^{3} is allelic to *upheld* mutations (DEAK *et al.* 1982; our unpublished data), that *sdby* is allelic to $fliI^{3}$ (MIKLOS *et al.* 1987), and that the mutation *fliF* (HOMYK and SHEPPARD 1977) is allelic to *gmp* (DEAK *et al.* 1982; our unpublished data), the original designations for these mutations have been retained in the present text.

More recent studies have shown that Sh^{101} (HOMYK and SHEPPARD 1977) is a doubly mutant strain. Through genetic recombination, a single mutant recombinant for the ether shaking phenotype has been isolated. The new single mutant, referred to as Sh^{101}, hops and flies normally when raised at 22° or 29°. No single mutant recombinants for the flightless mutation have been isolated. Cytogenetic mapping data (see below) and complementation tests show that this flightless mutation is, however, an allele of *hdp*, and it has been designated hdp^{101}. Since parallel studies have shown that Sh^{101} does not show interactions with any mutations in this study, all effects on flight are assumed to be due to the hdp^{101} mutation in this strain and it will be referred to simply as hdp^{101}.

Recent studies have shown that the mutation *fliH* (HOMYK and SHEPPARD 1977) fails to complement hdp^{101} and hdp^{2} for flightlessness, and has, accordingly, been redesignated hdp^{102}. Interestingly, hdp^{102} and hdp^{2} do show partial complementation, and hdp^{102}/hdp^{2} heterozygotes hop and fly 1–2 cm high and 2–5 cm distant, noticably better than either homozygote or hdp^{101}/hdp^{102} heterozygotes which are completely flightless.

Last, the mutation up^{101} was, erroneously, previously reported as semidominant for flightlessness (HOMYK, SZIDONYA and SUZUKI 1980). Thus, $up^{101}/+$ heterozygotes hop and fly as well as wild type (FEKETE and SZIDONYA 1979; Table 3). Rather, the mutation up^{whu}, reported previously as recessive, is semidominant for flightlessness.

Media: Drosophila cultures were grown on the semisynthetic medium described by CARPENTER (1950) and modified by E. WRIGHT (personal communication).

Behavioral characterization: Most of the mutations are nonconditional, *i.e.*, their phenotypes are independent of developmental temperature. *Inter se* crosses involving these mutations were incubated at 22°. Four mutations are developmentally temperature-sensitive (ts). Thus, $fliC^{2}$ is ts for recessive flightless and wing posture phenotypes, hdp^{101} is ts for recessive wing posture and semidominant flightless phenotypes and $fliA^{4}$ is ts for a recessive lethal phenotype. Last, *rsd* was found to be cold sensitive, the mutant heterozygotes capable of hopping and flying short distances (1–2 cm high, 4–6 cm distant) when raised at 29° but not when raised at 22°. Crosses involving these strains were incubated at both 22° and 29°.

Adult flies, aged 2–3 days at 22° on fresh media, were

TABLE 1

Chromosome rearrangements

Rearrangement	Genotype[a]		Cytology	Source
	Proximal	Distal		
Duplications				
$y;^2Y67g$			1A1;2B17[b]	K. WHITE
$Dp(1;3)w^{vco}$			2B17-C1;3C4-5[c]	Cal Tech
w^+Y			2D1-2;3D3-4[d]	Cal Tech
$Dp(1;3)sn^{13a1}$			6C11;7C9[b]	T. JOHNSON
$T(1;2)sn^{+72d}$			7A8;8A5[b]	Cal Tech
$Dp(1;4)r^+f^+$			13F;16A2[b]	T KAUFMAN
$Tp(1;3)f^+71b$			15A4;16C2-3[c]	Cal Tech
$Dp(2;3)osp^3$			35B3-4;36C11[f]	T. WRIGHT
Deletions				
Df(1)Pgd-kz			2D3;2F5[e]	N. PERRIMON
Df(1)JA52			2D3-4[e]	N. PERRIMON
Df(1)64c18			2E1-2;3C2[e]	Cal Tech
$Df(1)w^{258-48}$			3A9-B1;3C2-3[e]	Cal Tech
$Df(1)ct^{J6}$			6E1;7C1[e]	T. JOHNSON
$Df(1)ct^{4b1}$			7B2-4;7C3-4[e]	T. JOHNSON
Df(1)C246			11D;12A1-2[e]	Cal Tech
$Df(1)g^l$			12A;12E[e]	Cal Tech
Df(1)HA92			12A6-7;12D3[e]	Cal Tech
Df(1)N19			17A1;18A2[e]	Cal Tech
Df(2L)H20			36A6-7;36F1-2[g]	T. WRIGHT
Df(2L)H68			36B1-2;37B1[g]	T. WRIGHT
Reciprocal translocations				
T(1;Y)V59	$y^+ f$	$y\,w\,B^s$	11E[h]	Bowling Green
T(1;Y)B166	y^+	$y\,B^s$	12B[h]	Bowling Green
T(1;Y)B55	y^+	$y\,B^s$	16F1-4[i]	Bowling Green
T(1;Y)W32	y^+	$y\,w\,f\,B^s$	16F3-6[i]	Bowling Green
T(1;Y)V7	y^+	$y\,w\,f\,B^s$	16F5-8[i]	Bowling Green

[a] Genotype of proximal and distal elements of reciprocal *X;Y* translocations.
[b] CRAYMER and ROY (1980).
[c] LINDSLEY and GRELL (1968).
[d] JUDD, SHEN and KAUFMAN (1972).
[e] LINDSLEY and ZIMM (1987).
[f] SIMPSON (1983).
[g] MOGAMI *et al.* (1986).
[h] STEWART and MERRIAM (1973) and J. R. MERRIAM, personal communication.
[i] TANOYUE, FERRUS and FUJITA (1981).

examined for wing posture and for hopping and flying ability and running and climbing abilities in square half-pint milk bottles, approximately 5 cm x 13 cm in size. Flies were tested singly or in groups of five to ten with the bottles placed first in an upright position, then on their sides. The ability to fly to designated heights was recorded. Flight ability was divided into four categories. Flies which did not fly are referred to as flightless, and those which fly >8 cm high as normal, as judged by comparison to wild type. Flies which flew <4 cm are referred to as poor fliers, and those which fly between 4 and 8 cm high as moderate fliers.

Phenotypic interactions: Examination for mutant interactions was performed in two phases. In the first, males of each of the seven myosin heavy chain mutants were crossed to females of each of the other mutant strains in all pairwise combinations. All F_1 progeny, raised in shell vials at 22°, unless otherwise noted, were counted and the doubly heterozygous females examined for relevant phenotypes. In all cases involving an autosomal and an *X*-linked mutation, crosses were made between females bearing *X*-linked mutations and males bearing autosomal mutations, and males hemizygous for the *X*-linked mutation and heterozygous for the autosomal mutation examined also. With the exception of lethal or semilethal combinations, 30 or more flies were examined. Oregon-R wild-type flies and the appropriate single mutant heterozygous females from crosses to Oregon-R served as controls.

The second phase was conducted to examine possible interactions among the non-myosin mutations. Though not all mutant combinations were tested, those examined were chosen as being the most likely to reveal interaction on the basis of earlier crosses as the study progressed (see RESULTS).

Interactions involving alteration of myosin heavy chain gene dose: For interactions involving hypoploidy, *Df(2L)H20/CyO* (Table 1) males, carrying a deficiency of the *Mhc* locus, were crossed to females bearing each of the flightless mutations to be tested and to an Oregon-R control. Females heterozygous for the deficient chromosome were recognized as being non-Curly.

For interactions involving hyperploidy, *Ki* $Dp(2{:}3)osp^3/TM3$ males, carrying a copy of Mhc^+ in the insertional translocation (Table 1) were crossed to flightless mutant females and to an Oregon-R control. Progeny carrying three copies of Mhc^+ were recognized by the dominant *Ki* bristle phenotype of the translocation bearing chromosome.

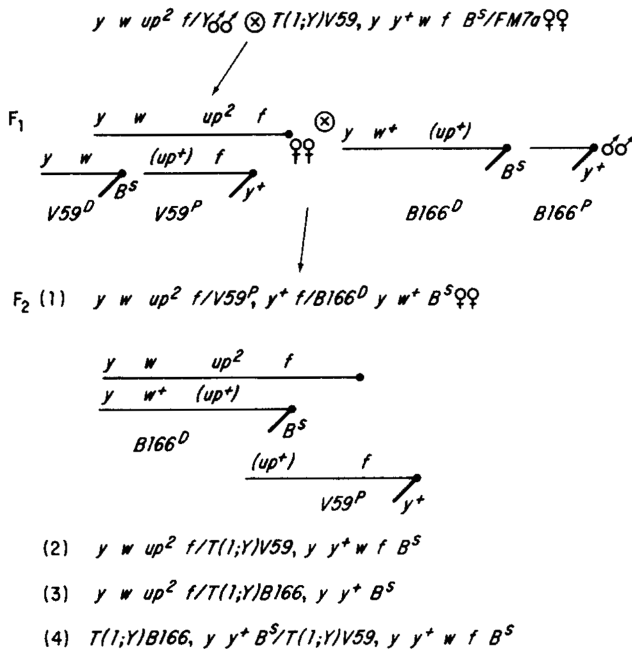

FIGURE 1.—Aneuploid mapping of up^2. Only relevant strains are shown. Diagram shows the constitution of *T(1;Y)V59* and *T(1;Y)B166*, including the probable location of up^+ (in parentheses) as determined in the present study. Thickened bar represents *Y* chromosomal elements.

In each case, the flight behavior of progeny heterozygous for a flightless mutation and $Dp(2{:}3)osp^3$ were compared to sibs heterozygous for the flightless mutation and the *TM3* balancer and to control progeny from a cross to Oregon-R.

Genetic mapping of interacting loci: Genetic mapping utilized a multiply marked *X*-chromosome stock obtained from Bowling Green. Mutant up^{101}/up^{101} and $y\ w\ hdp^2/FM7$ females were crossed, separately, to multiply marked, *y cv v f car/Y*, males. Heterozygous mutant females were allowed to mate with their sibs, then transferred to five fresh vials, 8 to 10 females/vial, on alternate days for 10 days. Recombinant males, collected separately from each set of vials, were mated singly to *C(1)DX, y w f/Y*, attached-X females. The recombinants cloned were $y\ cv\ v\ up^{101}$, $y\ cv\ v\ up^+$, $up^{101}\ f\ car$, and $up^+\ f\ car$ for the one mutation and $y\ cv\ v\ f\ hdp^2$, $y\ cv\ v\ f\ hdp^+$, $hdp^2\ car$ and $hdp^+\ car$ for the other. To insure independency, the three cloned chromosomes of each genotype tested were chosen from separate sets of vials.

Cytological mapping: Rearrangements used for cytological mapping of *up*, *hdp* and *fliA* mutations (Table 1) were chosen on the basis of their proximity to genetically determined positions and, in the case of *up*, to previously published results (DEAK *et al.* 1982).

In most cases, the mapping procedure was straightforward. A flightless mutant strain was crossed to a deletion or translocation bearing strain (Table 1) and the relevant progeny examined individually for flight behavior as described. Aneuploid mapping studies involving *up* and *hdp* mutations and reciprocal *X;Y* translocations required additional manipulations, and that the chromosome bearing the flightless mutation be marked with mutations with visible phenotypes. A summary of the schemes using aneuploidy to map *up* and *hdp* is described below. The chromosomal rearrangements used are described in Table 1. Note that the proximal and distal elements (denoted below with superscript P and D, respectively) of the reciprocal translocations are marked differently with *y*, y^+, *w*, *f* and B^s.

up: The chromosome containing up^2 was marked with the mutations *y* (yellow), *w* (white-eye) and *f* (forked bristle) by standard recombination techniques. Males of genotype $y\ w\ up^2\ f/Y$ were crossed to $T(1;Y)V59/FM7a,\ y^{31d}\ sc^8\ w^a\ v^{of}$ *B* females (Figure 1). F1 progeny $y\ w\ up^2\ f/T(1;Y)V59$ females, phenotypically white and forked, were selected and crossed to *T(1;Y)B166* males. The four possible classes of female progeny are shown in Figure 1, heterozygous deficient females being inviable (STEWART and MERRIAM 1973). Aneuploid females of class 1, carrying a duplication of the overlapping translocation elements and up^2 on their normal *X* chromosome were recognized as being phenotypically $w^+\ f$.

Parallel crosses using a strain homozygous *y w f/y w f*, and normal for up^+, were used to generate control flies, of the appropriate constitution, for comparisons of flight behavior.

hdp: Localization of *hdp* using aneuploidy employed the marked strains $y\ w\ hdp^{101}$, $y\ w\ hdp^2$ and $y\ hdp^3$, in crosses to strains carrying one of three reciprocal translocations, abbreviated here as *B55*, *V7*, and *W32*. For present purposes, it should be noted that each translocation contains a proximal element (superscript P) marked with y^+ (Table 1).

As an example, *T(1;Y)W32/FM7a* females were crossed to $y\ hdp^3/Y$ males. Two classes of aneuploid progeny result which are of interest here. Males carrying $y\ hdp^3$ on their normal *X* chromosome and $W32^P$, y^+ were recovered as y^+ non-Bar males. Females carrying the normal $y\ hdp^3$ chromosome, the *FM7a* balancer and the proximal translocation element $W32^P$, y^+, were recovered as $y^+\ w^+$ Bar-eyed females. These classes of aneuploid flies as well as *W32/Y* male and $y\ hdp^3/B55$ female progeny were examined individually for flight behavior. Crosses to the *V7* and *W32* translocation stocks as well as those involving hdp^2 and hdp^{101} and a $y\ w\ hdp^+$ control chromosome were treated identically.

Temperature-shift studies: Procedures for the synchronization of cultures at the egg and white prepupal stages and for conducting the temperature-shift and pulse studies have been described (TARASOFF and SUZUKI 1970; HOMYK and GRIGLIATTI 1983). For convenience, metamorphosis has been divided into five progressive stages: puparium (P1), pupae with unpigmented (P2), lightly pigmented (P3) or darkly pigmented (P4) eyes, and pupae with pigmented bristles and wing pads (P5).

Genetic mosaic analysis: A strain carrying an unstable ring-*X* chromosome, $In(1)w^{vC}/y\ w\ spl$ (HINTON 1955) was obtained from R. WILLIAMSON. Females carrying the unstable ring-*X* chromosome were crossed to males with an *X*-chromosome carrying $fliA^1$ and the visible cho^2 (eye color) and f^{36a} (bristle) mutations. All mosaic progeny were scored for their visible cuticle phenotype and their flight examined in square half-pint milk bottles on 3 successive days.

Mosaics which hopped and flew greater than 1 cm high and 3 cm distance, noticably better than either hemizygous or homozygous $fliA^1$ mutants, were scored as fliers. The remainder were scored as mutant. To facilitate comparison of the data with that obtained from previous studies of flightless mutations (HOMYK 1977; DEAK 1977; KOANA and HOTTA 1978), analysis of the data followed the procedure described by HOTTA and BENZER (1972).

RESULTS

Myosin heavy chain mutations: Molecular genetic studies localized the single, muscle specific myosin

TABLE 2

Phenotypic and cytological characterization of Mhc mutations

Genotype	Flight ability	Wing posture[a]	
		Normal	Abnormal
Bsh/ +	Poor	30	0
Ifm(2)1/ +	Moderate	30	0
Ifm(2)2/ +	Flightless	30	0
Ifm(2)3/ +	Flightless	0	30
Mhc^1/ +	Flightless	30	0
Nup/ +	Flightless	0	45
Stp/ +	Flightless	30	0
Df(2L)H20/ +	Flightless	30	0
Bsh/*Df(2L)H20*	Flightless	29	31
Bsh/*Df(2L)H68*	Not tested	30	0
Ifm(2)1/*Df(2L)H20*	Flightless	26	4
Ifm(2)1/*Df(2L)H68*	Normal	30	0
Mhc^1/ + ; $Dp(1;3)osp^3$/ +	Normal	30	0
Ifm(2)2/ + ; $Dp(1;3)osp^3$/ +	Normal	30	0
+ / + ; $Dp(1;3)osp^3$/ +	Normal	30	0

[a] Number of flies with normal or abnormal posture.

heavy chain gene in 36B (ROZEK and DAVIDSON 1983; BERNSTEIN *et al.* 1983). Seven flightless, and "putative" Mhc mutations, mapping in or around the region 36B, were chosen, on the basis of their having phenotypic differences, to examine their intergenic interactions with muscle defective mutations in other loci. It was hoped that the phenotypic differences reflect differences in each mutations effect on the Mhc protein, which would differentially affect the mutant proteins' intermolecular interactions. These mutations, including *Ifm(2)1*, *Ifm(2)2*, *Ifm(2)3*, Mhc^1, *Bsh*, *Stp* and *Nup*, were characterized genetically and phenotypically, based on judgment of three phenotypes in hemizygotes, homozygotes and heterozygotes. The phenotypes include lethality (or semilethality), abnormal wing posture, and flightlessness, in decreasing order of severity on the organism as a whole. This characterization was important to establish that these seven mutations are in the *Mhc* gene and to form a basis for recognizing intergenic interactions.

Previous studies (MOGAMI *et al.* 1986) have shown that the Mhc^+ gene is haploinsufficient for flight. Thus, flies heterozygous for *Df(2L)H20*, deficient for Mhc^+, are flightless, though they are fully viable, have normal wing postural and running and climbing phenotypes. That the haploinsufficiency is due to the Mhc^+ gene is supported by the observation that heterozygotes for an overlapping deficiency, *Df(2L)H68*, which deletes most of the genomic material deleted by *Df(2L)H20* except for Mhc^+ (MOGAMI *et al.* 1986; Table 1) hop and fly normally. Thus, the Mhc^+ gene and the "factor" haploinsufficient for flight map to the 4–9-band interval between the distal breakpoints of these two deletions. Furthermore, heterozygotes carrying the mutation Mhc^1, containing a 400-bp deletion in the *Mhc* gene (MOGAMI *et al.* 1986), are flightless. As expected, Mhc^1 behaves as a "null" mutation and has the same phenotype as the deficiency *Df(2L)H20*. Mhc^1 is homozygous lethal and heterozygotes have normal wing posture and normal running and climbing behaviors. Furthermore, the dominant flightless phenotype of Mhc^1 is completely covered in the presence of a second copy of Mhc^+, provided by the insertional translocation $Dp(2;3)osp^3$.

In general, *Ifm(2)3* is the most severe mutation, followed by *Nup*. Both mutations are dominant for indented thoraces and upheld wing postural phenotypes as well as flightlessness, even in the presence of $Dp(2;3)osp^3$, carrying a duplication of the Mhc^+ gene. This latter property suggests that they are antimorphic and that their mutant gene products interact extensively with normal gene products (OKAMOTO *et al.* 1986). The recessive lethal phenotype of *Ifm(2)3* is uncovered by *Df(2L)H20* but not by *Df(2L)H68* (MOGAMI *et al.* 1986; data not shown). Likewise, *Nup*/*Df(2L)H20* hemizygotes are semilethal, are very inactive, have weak mesothoracic legs which are generally folded beneath the thorax, and die prematurely after eclosion. *Nup*/*Df(2L)H68* and *Nup*/ + heterozygotes, on the other hand, are identical, having abnormal wing posture and flightless phenotypes but being fully viable.

Ifm(2)2/*Df(2L)H20* and *Stp*/*Df(2L)H20* hemizygotes are fully viable, flightless and have normal wing posture, and are, thus, identical to their respective heterozygotes. It is possible that *Ifm(2)2* is a null mutation that specifically affects the flight muscle specific *Mhc* isoform (MOGAMI *et al.* 1986). That it is recessive to two copies of Mhc^+ is consistent with this (Table 2). *Stp*, on the other hand, is dominant flightless in the presence of two Mhc^+ genes, and thus appears to be antimorphic.

Previous studies reported that *Ifm(2)1* and *Bsh* were also dominant flightless. In the present study, both mutations were judged to be semidominant for flightlessness, and thus, represent the weakest "putative" *Mhc* mutations included in this study. All *Ifm(2)1*/ + heterozygotes flew moderately well compared to controls (Table 2). *Bsh*/ + heterozygotes, on the other hand, displayed variable expressivity of phenotype. While a small fraction (20%) had indented thoraces and were flightless, the remainder had normal thoraces and flew, though poorly. The basis for this latter variation, as well as the differences between present and previous observations of the respective heterozygote phenotypes, has not been examined. In addition, *Bsh* has a recessive upheld wing postural phenotype.

The phenotypes of *Ifm(2)1* and *Bsh* are uncovered by *Df(2L)H20* but not by *Df(2L)H68*. Surprisingly, the flight behavior of *Bsh*/ + heterozygotes was not noticably improved by addition of the Mhc^+ dupli-

cation, *Dp(2;3)osp*^{3}. That *Bsh* is an antimorphic mutation is also supported by its "negative" intergenic interactions described below.

Genetic fine structure analyses were performed to map the dominant flightless mutations *Ifm(2)3*, *Mhc*^{1} and *Stp* with respect to *Ifm(2)2* and to determine whether it is reasonable that they could all be contained within the 20-kb *Mhc* sequence. As noted in MATERIALS AND METHODS the chromosome bearing *Ifm(2)2* also carried the flanking markers *b* and *pr*. No recombinants were found between *Ifm(2)2* and *Mhc*^{1}, among 12,875 progeny tested, and only one *b*+ recombinant was detected from the cross involving *Ifm(2)3*, among 13,563 progeny tested, indicating that *Ifm(2)3* is distal to *Ifm(2)2*. Considering that only half of the recombinants would have been detected and that 1% recombination approximates 275 kb of DNA (KIDD, LOCKETT and YOUNG 1983), these studies suggest that *Mhc*^{1} and *Ifm(2)3* are within 4 kb of *Ifm(2)2*, and that all three mutations could be accommodated into the 20-kb *Mhc* gene. Failure to detect recombinants between *Stp* and *Ifm(2)2* among 4,386 progeny is also consistent with *Stp* being within the gene.

In summary, cytological mapping studies have shown that *Bsh*, *Ifm(2)1*, *Ifm(2)3* and *Nup*, as well as *Mhc*$^+$, map to a 4–9-band interval, between the distal breakpoints of *Df(2L)H68* and *Df(2L)H20*, while genetic mapping studies have shown that *Stp*, *Mhc*^{1}, *Ifm(2)3* and *Ifm(2)2* map to a recombinationally short interval that could well be accommodated in the *Mhc* gene sequence. Although the interpretation of the results of complementation tests are complicated by possible intergenic interactions, some additional studies support the notion that the above seven mutations are in the *Mhc* gene. First, no interaction was found between lethal mutations defining six additional complementation groups in the relevant 4–9-band interval (STEWARD and NÜSSELIN-VOLHARD, 1986) and *Mhc* mutations. Second, a genomic walk extending for 50 kb on either side of the *Mhc* gene revealed *five* separately transcribed regions, none of which is muscle specific (E. GEORGE, personal communication).

Genetic interactions between flight defective and myosin heavy chain mutations: An initial survey was conducted, using mutations in a gene coding for a known myofibrillar protein, to test the hypothesis that interaction between myofibrillar proteins would be revealed in genetic interactions between mutations in the genes encoding these proteins. Thus, pairwise combinations were first made between each of the seven *Mhc* mutations and the remaining flight defective mutations, and the phenotypes of doubly mutant flies examined. In most cases, two nonallelic mutations were judged to interact on the basis that doubly heterozygous females had a more extreme phenotype than either of the respective single heterozygotes. This was clearest in cases in which two recessive flightless mutations interacted to cause flightlessness. In other cases, evidence for interaction was determined on the basis that males hemizygous for an *X*-linked mutation and heterozygous for an autosomal mutation had more extreme phenotypes than the respective singly mutant males. For evaluation purposes, the phenotypes that resulted from genetic interaction, in order of increasing severity, were flightlessness, abnormal wing posture, abnormalities in walking or climbing, and lethality.

Several types of interaction occurred involving *Mhc* mutations and mutations in other genes. The most extreme result of interaction was lethality. The *X*-linked mutations *up*^{101}, *int*^{3} and *hdp*^{2} were lethal in heterozygous combination with *Ifm(2)3* (Table 4). Thus, the fraction of doubly heterozygous mutant females is less than 10% that of either single heterozygote (compare column 1 with columns 2 and 3, Table 4). Analysis of relevant single heterozygotes from experimental and control crosses revealed no reduced viability (Table 4).

As expected, from results of the doubly heterozygous females, males hemizygous for *int*^{3}, *up*^{101} or *hdp*^{2} and heterozygous for *Ifm(2)3* were also inviable. Additionally, males hemizygous for *up*^{x} and heterozygous for *Ifm(2)3* were inviable. In agreement with previous results on *up* and *hdp* mutations (DEAK *et al.* 1982), the *int*^{3} and *hdp*^{2} mutations reduce viability in hemizygous males (Table 4).

All other doubly mutant combinations involving *Ifm(2)3* had normal viability. Notably, males hemizygous and females heterozygotes for either *up*^{whu} *up*^{2}, *up*^{3}, *hdp*^{3}, *hdp*^{4}, *hdp*^{5}, *hdp*^{101} or *hdp*^{102} and heterozygous for *Ifm(2)3* had normal viability (not shown). Furthermore, none of the viable doubly mutant combinations had a more severe phenotype than did *Ifm(2)3*/+ heterozygotes.

Comparisons of the several classes of females (Table 4) suggest that females doubly heterozygous for *up*^{101}, *up*^{x}, *int*^{3} or *hdp*^{2} and either *Nup* or *Bsh* have normal viability. Males hemizygous for *int*^{3}, *hdp*^{2} or *up*^{101} and either *Bsh* or *Nup*, however, were absent or greatly reduced in number (Table 4), and the rare "escapers" had "gnarled" legs, walked poorly and died within 2 days of eclosion.

Judged from the behavioral phenotype, it is also evident that *int*^{3}, *up*^{101}, *up*^{x} and *hdp*^{2} interact with *Bsh*. The doubly heterozygous females were completely flightless and had abnormal wing posture (Table 5). A similar phenotypic evaluation could not be made for *Nup* since this mutation is dominant for flightlessness and abnormal wing posture. Among the other allelic combinations, only *up*^{2}/+; *Bsh*/+ and *hdp*^{101}/+; *Bsh*/+ mutants showed evidence of interaction, through affected wing posture (Table 5).

The results of crosses involving the mutation

TABLE 3

Behavior of single mutant homozygotes and heterozygotes

Mutation	Homozygotes			Heterozygotes		
		Wing posture[b]			Wing posture[b]	
	Flight behavior[a]	Normal	Abnormal	Flight behavior[a]	Normal	Abnormal
ewg	Flightless	2	28	Normal	30	0
fliA^{1}	Flightless	30	0	Normal	30	0
fliA^{2}	Flightless	30	0	Poor	30	0
fliA^{3}	Flightless	30	0	Normal	30	0
fliA^{4}	Flightless	30	0	Normal	30	0
fliC^{2}[c]	Flightless	0	30	Normal	30	0
flil^{3}	Flightless	30[d]	0	Normal	30	0
sdby	Flightless	30[d]	0	Normal	30	0
fliF	Flightless	30	0	Normal	30	0
gmp^{2}	Flightless	30	0	Normal	30	0
flw^{3}	Flightless	0	30	Normal	30	0
gnd^{1}	Flightless	30	0	Normal	30	0
hdp^{2}	Flightless	0	30	Normal	30	0
hdp^{3}	Flightless	0	30	Flightless	30	0
hdp^{4}	Flightless	0	30	Flightless	30	0
hdp^{5}	Flightless	0	30	Flightless	30	0
hdp^{101}	Flightless	30	0	Normal	30	0
hdp^{101}[c]	Flightless	6	24	Poor	30	0
hdp^{102}	Flightless	30	0	Normal	30	0
Ifm(3)3	Flightless	30	0	Poor	30	0
Ifm(3)7	Flightless	30	0	Flightless	30	0
rsd	Flightless	0	30	Flightless	30	0
sr	Flightless	30	0	Normal	30	0
int^{3}	Flightless	0	30	Normal	30	0
up^{2}	Flightless	0	30	Flightless	30	0
up^{3}	Flightless	0	30	Flightless	30	0
up^{101}	Flightless	0	30	Normal	30	0
up^{x}	Flightless	0	30	Normal	30	0
up^{whu}	Flightless	23	7	Moderate	30	0

[a] Behavior comparable to the Oregon-R controls is denoted as normal.
[b] Number of flies with normal or abnormal posture. No distinction was made between mutants carrying wings in an upheld or a ventrolateral posture. Phenotype is age dependent in some strains. Thus, newly enclosed *up*^{101}/*up*^{101} mutants have normal wing posture but develop abnormal posture during first 24 hr.
[c] Raised at 29°.
[d] Mutant stocks have normal posture. A variable fraction of males from outcrosses of mutant females to Oregon-R males have abnormal posture.

Ifm(2)1 revealed a slightly different pattern of interactions. As noted, *Ifm(2)1*/+ heterozygotes hop and fly moderately well, suggesting that *Ifm(2)1* is weakly semidominant for flightlessness. The mutations *int*^{3}, *up*^{101} and *up*^{x} showed strong interaction with *Ifm(2)1*, as with the other *Mhc* mutations described above. The respective double heterozygotes did not hop and fly, though they did have normal wing posture (Table 5). Likewise, a majority of doubly heterozygous *Ifm(2)1*/+; *rsd*/+ mutants had abnormal wing posture in contrast to either single heterozygote (Tables 2, 3 and 5). Surprisingly, *hdp*^{2} showed no evidence of deleterious interaction and *hdp*^{2}/+; *Ifm(2)1*/+ females hopped and flew as well as *Ifm(2)1*/+ single heterozygotes, while *hdp*^{101}/±; *Ifm(2)1*/+ and *hdp*^{102}/+; *Ifm(2)1*/+ heterozygotes hopped and flew poorly (Table 5).

No interactions were observed for the remaining combinations of *hdp* or *up* alleles and *Bsh*, *Nup* or *Ifm(2)1*. Furthermore, no interactions were noticed between *Mhc*^{1}, *Stp* or *Ifm(2)2* and any *hdp*, *fliA* or *up* mutations examined in this study. Since the latter three Mhc mutations are dominant flightless, interaction would have been noted only if it produced the more severe wing posture, leg coordination or lethal phenotypes. Likewise, no interactions were noted between the *Mhc* mutations and any of the remaining mutations. Thus, altogether, mutations in 3 of 12 genes examined showed intergenic interaction with one or more *Mhc* mutations.

Interactions involving non-myosin heavy chain gene mutations: Considering that some intergenic interactions were observed involving *Mhc* gene mutations, selected doubly mutant combinations among

TABLE 4

Lethal interactions between *Mhc* and non-*Mhc* mutations

Cross	Females[a]				Males[a]			
	$M^1/+$; $M^2/+$	$M^1/+$; $CyO/+$	$FM6/+$; $M^2/+$	$FM6/+$; $CyO/+$	M^1/Y; $M^2/+$	M^1/Y; $CyO/+$	$FM6/Y$; $M^2/+$	$FM6/Y$; $CyO/+$
$hdp^2/FM6$; +/+ ♀⊗ +/*Y*; *Ifm(2)3/CyO* ♂	0	67	53	63	0	53	34	54
$int^3/FM6$; +/+ ♀⊗ +/*Y*; *Ifm(2)3/CyO* ♂	1	149	119	132	0	78	111	112
up^{101}/up^{101} ♀⊗ +/*Y*; *Ifm(2)3/CyO* ♂	15	150			0	100		
$up^x/FM6$; +/+ ♀(x) +/*Y*; *Ifm(2)3/CyO* ♂	24	57	59	46	0	3	33	33
$hdp^2/FM6$; +/+ ♀⊗ +/*Y*; *Bsh/Bsh* ♂	113		121		3		83	
$int^3/FM6$; +/+ ♀⊗ +/*Y*; *Bsh/Bsh* ♂	66		81		1		51	
up^{101}/up^{101}; +/+ ♀⊗ +/*Y*; *Bsh/Bsh* ♂	198				43			
$hdp^2/FM6$; +/+ ♀⊗ +/*Y*; *Nup/CyO* ♂	37	49	40	47	0	29	13	29
$int^3/FM6$; +/+ ♀⊗ +/*Y*; *Nup/CyO* ♂	130	146	135	144	0	77	88	103
up^{101}/up^{101}; +/+ ♀⊗ +/*Y*; *Nup/CyO* ♂	36	37			0	26		
$up^x/FM6$; +/+ ♀(x) +/*Y*; *Nup/CyO* ♂	39	34	28	29	0	10	10	10
Controls:								
Oregon-R ♀⊗ +/+; *Ifm(2)3/CyO* ♂	49	40			52	26		
Oregon-R ♀⊗ +/+; *Bsh/Bsh* ♂	102				110			
Oregon-R ♀⊗ +/+; *Nup/CyO* ♂	45		28		51		53	
$hdp^2/FM6$; +/+ ♀⊗ Oregon-R ♂	27		36		16		17	
$int^3/FM6$; +/+ ♀⊗ Oregon-R ♂	32		31		23		26	
up^{101}/up^{101}; +/+ ♀⊗ Oregon-R ♂	29					23		
$up^x/FM6$; +/+ ♀(x) Oregon-R ♂	47		38		22		26	

[a] M^1 refers to any *X*-linked mutation or "+" allele as present in the cross. M^2 refers to any second chromosomal Mhc mutation or "+" allele as present in the cross. *FM6* and *CyO* are balancer chromosomes.

the non-*Mhc* mutations were examined. During the survey, mutations in one additional gene, *fliA* were found to interact with a mutation in *hdp*. Because of this, and because interactions had been detected between *Mhc* mutations and alleles of the *up* (*int*), *hdp*, and *rsd* (*act88F*) loci, all doubly mutant combinations between mutations in these four genes were surveyed. Following this, *rsd* and the recessive mutations int^3, up^{101}, up^x, hdp^2, hdp^{101}, hdp^{102}, $fliA^1$, $fliA^3$ and $fliA^4$, were likewise examined in combination with each of the remaining behavioral mutations in the study.

No reduction in viability, attributable to interaction, was detected among the remaining mutant combinations. Notable interactions involving mutations in four loci, however, were evident, judging from wing posture and/or flightless phenotypes.

The most extensive set of interactions occurred between *hdp* and *up* mutations (Table 6). Notably int^3 +/+ hdp^2, up^{101} +/+ hdp^2 and up^x +/+ hdp^2 het-

TABLE 5

Myosin heavy chain mutant behavioral interactions

Genotype	Flight behavior	Wing posture	
		Normal	Abnormal
hdp^{101}/Y; $Bsh/+$	Flightless	37	23
$hdp^{2}/+$; $Bsh/+$	Flightless	1	29
$int^{3}/+$; $Bsh/+$	Flightless	0	66
$up^{2}/+$; $Bsh/+$	Flightless	17	13
$up^{101}/+$; $Bsh/+$	Flightless	23	27
$up^{x}/+$; $Bsh/+$	Flightless	21	9
$hdp^{101}/+$; $Ifm(2)1/+$	Poor	30	0
$hdp^{102}/+$; $Ifm(2)1/+$	Poor	30	0
hdp^{102}/Y; $Ifm(2)1/+$	Flightless	39	21
$int^{3}/+$; $Ifm(2)1/+$	Flightless	15	3
$Ifm(2)1/+$; $rsd/+$	Flightless	14	24
$up^{101}/+$; $Ifm(2)1/+$	Flightless	30	0
$up^{x}/+$; $Ifm(2)1/+$	Flightless	30	0

TABLE 6

Non-myosin mutant behavioral interactions

Genotype	Flight behavior	Wing posture	
		Normal	Abnormal
$fliA^{3}$ +/+ hdp^{2}	Flightless	30	0
$fliA^{4}$ +/+ hdp^{2}	Flightless	123	0
$EA82$ +/+ hdp^{2}	Flightless	30	0
$EA82^{HC207}$ +/+ hdp^{2}	Flightless	30	0
int^{3} +/+ hdp^{2}	Flightless	0	30
int^{3} +/+ hdp^{3}	Flightless	10	30
int^{3} +/+ hdp^{4}	Flightless	5	63
int^{3} +/+ hdp^{5}	Flightless	0	30
int^{3} +/+ hdp^{101}	Moderate	60	0
int^{3} +/+ hdp^{102}	Moderate	30	0
up^{101} +/+ hdp^{2}	Flightless	0	30
up^{101} +/+ hdp^{3}	Flightless	25	5
up^{101} +/+ hdp^{4}	Flightless	16	14
up^{101} +/+ hdp^{5}	Flightless	11	23
up^{101} +/+ hdp^{101}	Poor	47	0
up^{101} +/+ hdp^{102}	Poor	30	0
$up^{2}/+$; $rsd/+$	Flightless	21	9
$up^{101}/+$; $rsd/+$	Flightless	0	30
$up^{101}/+$; $rsd/+$ (29°)	Flightless	30	0
up^{x} +/+ hdp^{2}	Flightless	1	28
up^{x} +/+ hdp^{4}	Flightless	13	14
up^{x} +/+ hdp^{5}	Flightless	7	5
up^{x} +/+ hdp^{101}	Poor	37	5

erozygotes were flightless and had abnormal wing posture. Though not as striking, a similar interaction greatly reduced flight ability in int^{3} +/+ hdp^{101}, up^{101} +/+ hdp^{101}, int^{3} +/+ hdp^{102}, up^{101} +/+ hdp^{102} and up^{x} +/+ hdp^{101} heterozygotes.

Interactions involving dominant flightless mutations at these two loci were also observed. Thus, a major portion of int^{3} +/+ hdp^{3}, int^{3} +/+ hdp^{4}, int^{3} +/+ hdp^{5}, up^{101} +/+ hdp^{4}, up^{101} +/+ hdp^{5}, up^{x} +/+ hdp^{4} and up^{x} +/+ hdp^{5} heterozygotes and a small fraction of up^{101} +/+ hdp^{3} heterozygotes had abnormal wing posture (Table 6). Interestingly, all double mutants involving *hdp* mutations and the dominant up^{2} and up^{3} alleles had normal wing posture (not shown).

The *fliA* mutation tested in the first series of crosses, $fliA^{4}$, interacted with hdp^{2} to cause flightlessness. Following this observation, examination of three other alleles, $fliA^{1}$, $fliA^{2}$ and $fliA^{3}$, in combination with all other mutations, revealed another case of strong interaction with hdp^{2}. Thus, $fliA^{3}$ +/+ hdp^{2} heterozygotes were flightless (Table 6).

The last combination of nonallelic interactions involves *raised* (*rsd*) in combination with *up* (*int*) mutations. The present study, confirming an earlier report (Deak *et al.* 1982), revealed an interaction in $up^{2}/+$; $rsd/+$ heterozygotes that causes abnormal wing posture. Interestingly, a similar interaction produced a wing posture defect in $up^{101}/+$; $rsd/+$ heterozygotes raised at 22° but not when raised at 29° (Table 6). No observable interaction occurred between *rsd* and the remaining alleles of *up*, including int^{3} and up^{x}, nor between any *up* mutation and $Ifm(3)7$.

Last, no interactions were detected in heterozygotes involving hdp^{101} raised at 29°, other than those observed in heterozygotes raised at 22°. No interactions involving *ewg*, $fliC^{2}$, flw^{3}, gmp^{2}, gnd^{2}, $Ifm(3)3$, *sdby*, $fliI^{3}$ or *sr* in combination with one another, with myosin heavy chain mutations, *rsd* or with recessive mutations at the other loci included in this study were observed.

Altogether, intergenic interactions were detected between mutations in five genes, including the *Mhc* gene, among the mutations in 13 genes tested. As will be discussed, the pattern of interactions shows evidence of both intragenic and intergenic specificities.

Interactions involving altered gene dose: In view of the hypothesis being tested, which is that the observed interactions reflect biochemical interactions between many different and stochiometrically related gene products in the highly organized myofibrillar complex, it follows that perturbations in the relative amounts of functional gene products could also be an important factor. To test this possibility more directly, a deletion and an insertional translocation of Mhc^{+} and an insertional translocation of $fliA^{+}$ were tested for "interaction" in combination with selected mutations in separate genes. Thus, $Df(2L)H20$ and $Dp(2;3)osp^{3}$, providing tests for one and three copies of Mhc^{+} respectively, were examined in combination with each of the mutations $fliA^{1}$, $fliA^{2}$, $fliA^{3}$, $fliA^{4}$, hdp^{101}, hdp^{102}, hdp^{2}, $fliC^{2}$, *sdby*, $fliI^{3}$, *rsd*, up^{whu}, int^{3}, up^{101} and up^{x}. Since $Df(2L)H20$ is flightless, interaction would have been detected only if it produced more severe wing postural or lethal phenotypes. No such cases were observed (not

TABLE 7

Interactions involving altered Mhc$^+$ dose

Genotype	Flight behavior	Wing posture	
		Normal	Abnormal
$+/+$; $Dp(2;3)osp^3/+$	Normal	30	0
$hdp^2/+$; $Dp(2;3)osp^3/+$	Moderate	30	0
$int^3/+$; $Dp(2;3)osp^3/+$	Flightless	30	0
$up^{101}/+$; $Dp(2;3)osp^3/+$	Flightless	30	0
$up^x/+$; $Dp(2;3)osp^3/+$	Flightless	30	0

shown). Although the appropriate wild type control carrying three copies of Mhc^+ hopped and flew normally, mutants that are heterozygous for one of the recessive mutations up^{101}, up^x or int^3 and which carry three copies of Mhc^+ are flightless (Table 7). Likewise mutants heterozygous for the recessive mutation, hdp^2, and which carry three Mhc^+ gene copies have reduced flight ability (Table 7). Thus, the myofibrillar structure of mutants heterozygous for either up^{101}, int^3, up^x or hdp^2 are more sensitive than appropriate controls to increases in Mhc^+ gene dose. This suggests that there is a strict stoichiometric relationship between the products of these three genes, and that a duplication of Mhc^+ in heterozygous combination with some recessive mutations in either *hdp* or *up* (*int*) alters the relationship beyond an optimum threshold for myofibrillar assembly. Similar combinations involving each of the remaining ten mutations tested had no effect.

Thus far, only hdp^2 has been tested in combination with three copies of the *fliA* locus, using $Dp(1;3)w^{vco}$, and no evidence for interaction was observed.

Interactions with mutations of the triplolethal locus: The discovery that the interacting mutations map to haploinsufficient regions (see below) suggested that further interactions might be found involving mutations mapping to other dosage sensitive regions. A large number of mutations in the triplolethal locus (*Tpl*) have been isolated and characterized (KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980). This locus is unique in Drosophila, in that it is both haplo- and triplolethal. Genetic studies have led to the isolation of at least two classes of mutations at the *Tpl* locus that allow survival of flies carrying three copies of the region. The first, apparently stronger alleles, are themselves heterozygous lethal and behave as "null" alleles or deletions. The second, apparently weaker alleles, are recessive lethals, fully viable as heterozygotes. Three mutations in the latter class, Tpl^{10}, Tpl^{17} and Tpl^{38} (ROEHRDANZ and LUCCHESI 1980), were each tested in combination with up^{101}, up^x, int^3 and hdp^2. None of these *Tpl* mutations affected flight in single heterozygotes, and no interaction, regarding flight or viability, was detected with the flight defective mutations tested.

Genetic mapping of phenotypic interactions: To examine the possibility that the observed interactions are due to unrelated mutations common to the different strains, up^{101} and hdp^2 were chosen for genetic mapping studies. These mutations map in the *v-f* and *f-car* intervals, respectively, on the *X* chromosome. Three independent recombinants of each of the four recombinant classes were cloned in each case (see MATERIALS AND METHODS) from crosses involving a multiply marked, *y cv v f car*, *X* chromosome. Recombinant chromosomes from crosses involving up^{101} were tested with hdp^2 and $Ifm(2)1$. Those involving hdp^2 were tested with $fliA^4$, up^{101} and $Ifm(2)1$. All recombinant chromosomes carrying the up^{101} or hdp^2 mutations showed the same interactions as did the parental mutant chromosomes, while recombinant chromosomes carrying the normal alleles, up^+ or hdp^+, did not. These data support the conclusion that the up^{101} and hdp^2 mutations are responsible for the genetic interactions.

Cytological mapping studies: Previous studies have localized mutations in the myosin heavy chain gene to 36B (MOGAMI *et al.* 1986) and two mutations in *rsd*, one of which is in the flight muscle specific actin gene in 88F (MAHAFFEY *et al.* 1985). In preparation for future genetic and molecular genetic studies, the cytological localization of each of the remaining genetically interacting loci was also determined.

hdp: Initial studies showed that hdp^{101} was not covered by $Dp(1;4)r^+$ or $Tp(1;3)f^+71b$ and not uncovered by $Df(1)N19$ (data not shown), defining the locus to the region 16C2,3 to 17A1 (Table 1). Three *hdp* alleles, hdp^2, hdp^3 and hdp^{101} were used in conjunction with three reciprocal *X;Y* translocations to define the location of the locus more precisely.

The scheme used to generate and recognize relevant aneuploid heterozygotes is detailed in Materials and Methods. The three translocations used, abbreviated *B55*, *W32* and *V7*, have breakpoints in 16F (Table 1) and consist of distal and proximal elements, denoted here by the superscripts D and P, respectively. Males hemizygous for any of the three translocations and females heterozygous for any of the three translocations and a control chromosome, hopped and flew as well as an Oregon-R control. Furthermore, $hdp^{101}/B55$, $hdp^{101}/W32$, $hdp^{101}/V7$, $hdp^2/B55$ and $hdp^2/V7$, as well as $hdp^{101}/+$ and $hdp^2/+$ heterozygotes, hopped and flew as well as Oregon-R, showing that the hdp^+ gene on each of the translocation chromosomes covers the recessive phenotypes of these mutations. Likewise, $hdp^3/B55$, $hdp^3/W32$ and $hdp^3/V7$ females retain the dominant flightless phenotype but have normal wing posture.

Males carrying the control (*y w* hdp^+) chromosome and a proximal element of any one of the translocations hopped and flew moderately well. All males of the constitution $hdp^{101}/W32^P$ hopped and flew well

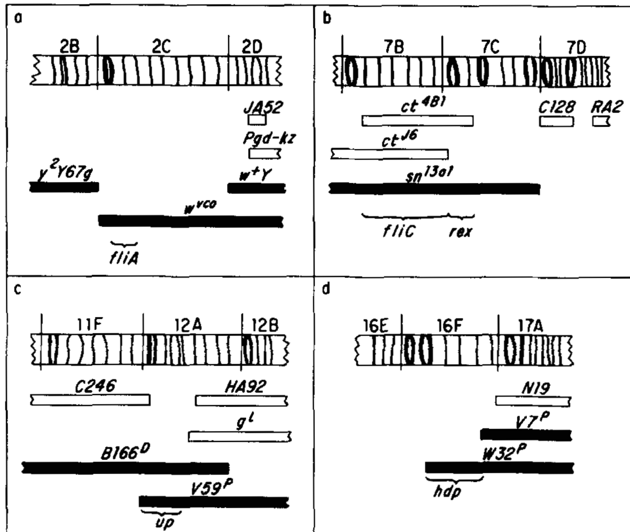

FIGURE 2.—Cytological localization of *fliA, hdp, up, fliC* and *rex* complementation groups. *Solid bars* represent extents of duplications or translocations. *Hollow bars* represent extents of deletions. See Table 1 and text for details.

while those with the constitution $hdp^{101}/V7^P$ hopped less than 1 cm and did not fly. Likewise, most $hdp^2/B55^P$ males hopped and flew moderately well and all had normal wing posture. By comparison, all $hdp^2/V7^P$ males had an upheld phenotype and none flew, thus behaving like hemizygous hdp^2/Y males.

Cytological studies involving hdp^3 agree with the above results. Surprisingly, $hdp^3/W32^P$ males have normal wing posture and hop and fly moderately well, the hdp^+ allele carried in $W32^P$ partially overcoming the dominant hdp^3 phenotype in males. Furthermore, $hdp^3/FM7a/W32^P$ females, apparently carrying two copies of hdp^+ and one of hdp^3, hopped and flew as well as wild type. On the other hand, $hdp^3/V7^P$ males did not fly and had abnormal wing posture; and $hdp^3/FM7a/V7^P$ females did not fly. Altogether, these data define hdp^+ to the haplolethal region 16F3,4-16F6,8 (TANOUYE, FERRUS and FUJITA 1981), between the breakpoints of the *W32* and *V7* translocations (Figure 2).

up: Initial studies showed that up^{101} is not uncovered by *Df(1)C246, Df(1)*g^l or *Df(1)HA92*, spanning all but possibly a short segment within the 11D to 12E region (Table 1). This agrees with DEAK *et al.* (1982) who proposed that *up* is located in a short, non-overlapping segment between *Df(1)C246* and *Df(1)HA92* or *Df(1)*g^l.

The fact that no deletions span this region can be explained by the possibility that it contains a haplolethal region, shown by STEWART and MERRIAM (1973) to occur in the region 11E to 12B. To substantiate the possibility that up^+ is located in the haplolethal region, the dominant flightless mutant up^2 was used in a series of crosses to the overlapping translocations (STEWART and MERRIAM 1973; J. MERRIAM, personal communication) used to define the haplolethal region (see MATERIALS AND METHODS and Figure 1).

Females heterozygous for up^2 and either *T(1;Y)B166* or *T(1;Y)V59* have normal wing posture and are flightless, identical to $up^2/+$ heterozygous controls. Aneuploid females, carrying up^2 on their normal chromosome and a duplication of the 11E to 12B region ($up^2/B166^D/V59^P$), hopped and flew as well as the wild type controls. Thus, two copies of up^+ are sufficient to overcome the dominant flightless phenotype of up^2. In view of the fact that this region is diplolethal in males (STEWART and MERRIAM 1973), it is noteworthy that aneuploid females, from control crosses, carrying three copies of up^+, showed no debilitation regarding flight performance. Altogether, these results suggest that the *up* locus occurs in a short interval in 12A, between the ends of *Df(1)C246* and *Df(1)HA92* or *Df(1)*g^l (Figure 2) and that this interval is also haplolethal.

fliA: mutations in *fliA* map to the distal end of the *X* chromosome. Initial studies showed that the flightless phenotype of $fliA^4$ was not covered by y^2Y67g, placing the locus proximal to 2B17 (Table 1). Subsequent studies revealed that the flightless phenotype is covered by *Dp(1;3)*w^{vco} but not by w^+Y, placing $fliA^+$ in the haplolethal interval 2B17 to 2D1.

In agreement with the duplication mapping, $fliA^4$ lethal and flightless phenotypes were not uncovered by *Df(1)Pgd-kz*, deleting 2D3,4 to 2F5 (Table 1), or by *l(1)JA52*, a cytologically normal mutation which fails to complement mutations in three complementation groups localized between 2D1,2 and 2D3,4 (PERRIMON, ENGSTROM and MAHOWALD 1985).

$fliA^4$ was also found to complement mutations in seven of eight lethal complementation groups in the 2C3-2E1 interval (see MATERIALS AND METHODS). Regarding the exception, $fliA^4$ failed to complement *l(1)EA82* and *l(1)EA82*^{HC207} for lethality, when raised at 29°. Likewise, the respective heterozygotes were flightless and semilethal when raised at 22°. Heterozygotes for $fliA^1$, $fliA^2$, or $fliA^3$ and *EA82* or $EA82^{HC207}$ were fully viable but flightless. As noted (PERRIMON, ENGSTROM and MAHOWALD 1985), $EA82^{HC207}$ is an inversion with a breakpoint in 2C3, localizing the *fliA* gene in or near this band (Figure 2).

The identification of two additional lethal mutations in *fliA* gave the opportunity to further examine the specificity of interaction of *fliA* and *hdp* mutations. All doubly heterozygous mutant combinations between *EA82* and $EA82^{HC207}$ and the flightless mutations *fliC*, hdp^2, hdp^{101}, hdp^{102}, up^{101}, up^{whu}, *rsd*, *Ifm(2)1*, *Ifm(2)3*, *Bsh* and *Nup* were examined. None of the combinations affected viability and only two doubly mutant combinations affected flight behavior. Mutant *EA82* $+/+$ hdp^2 and $EA82^{HC207}$ $+/+$ hdp^2

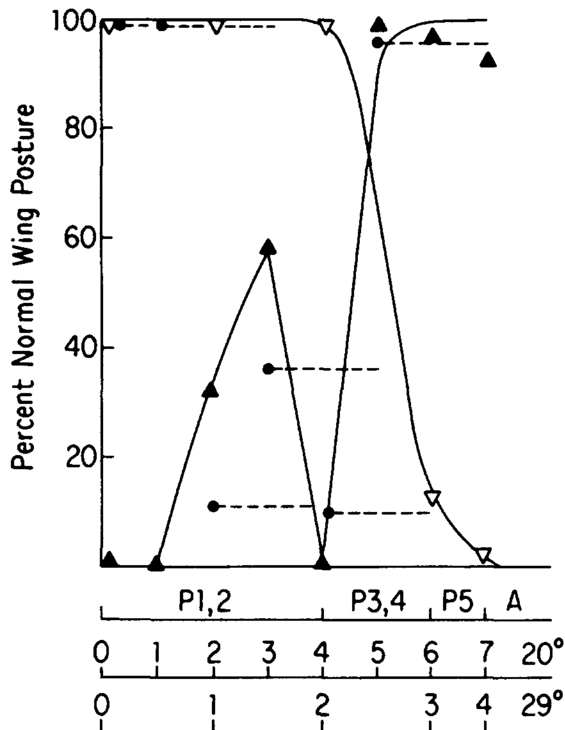

FIGURE 3.—Temperature-shift experiment to determine TSP for wing posture phenotype of *hdp*[101] males. Abcissa represents fraction of mutants with normal wing posture. Ordinate represents developmental stage. Times at which cultures were shifted up to 29° (▲), down to 22° (▽), or pulsed up to 29° (●) for 24 hr are indicated.

heterozygotes were completely flightless but had normal wing posture (Table 6). This further supports the observed specificity of interaction between the *hdp*[2] mutant product and *fliA* mutant products.

fliC: the localization of all five genetically interacting loci, affecting muscle development, to haploinsufficient regions is significant. For this reason, cytological analysis of *fliC*, which shows no interactions but which maps in the vicinity of the haplolethal region in 7C (LEFEVRE and JOHNSON 1973; STEWART and MERRIAM 1973) was undertaken. This showed that *fliC* is covered by $Dp(1;2)sn^{+72d}$ and $Dp(1;3)sn^{13a1}$ and uncovered by $Df(1)ct^{4b1}$ and $Df(1)ct^{J6}$, placing it in the interval 7B2-4 to 7C1, just distal to the haplolethal region (Figure 2).

Temperature-shift studies of *hdp*[101]: The developmentally ts wing posture phenotype of *hdp*[101] made determination of a temperature sensitive period (TSP) possible. Initial shift studies, using cultures synchronized at the egg stage, suggested that the TSP for the recessive wing postural phenotype is limited to pupal development (not shown). Refinement of the TSP was made using cultures synchronized at the white puparium stage (BODENSTEIN 1965; see MATERIALS AND METHODS).

All males, incubated at 29° throughout pupal development, held their wings in a fully vertical position. When treated likewise, approximately 70% of the females held their wings in a near vertical position and 30% held them in an intermediate position or alternated between this and a normal posture. In spite of the increased expressivity in males, the TSP profiles for wing posture were essentially identical in three independent experiments in both sexes, and only data for males is presented in Figure 3.

In the downshift experiment, wing posture is normal in cultures shifted on or before day two following pupariation, at the early P3 stage (see MATERIALS AND METHODS). The fraction with normal posture dropped to zero by day four, at which time adults began to eclose.

Results of the upshift experiment were more complicated. No mutants shifted at pupariation or one day past pupariation held their wings normally. The fraction with normal wing posture, however, rose to 55% for cultures shifted on day three (early P3), then dropped to zero for those shifted on day 4, before rising to 100% for mutants shifted on day 6 (P5).

The biphasic nature of the curve in the upshift experiments suggests that two processes, with different TSPs, may be affected by the mutation. Perhaps associated with this possibility, the thoraces of 50% of the females and 70% of the males, shifted or pulsed up on day 4, were severely intented. No indentions were observed on mutants shifted or pulsed at other times.

The TSP defines the developmental stage(s) during which ts mutations exert their effect and, in some instances, reflects the time of function and/or synthesis of the relevant gene product (SUZUKI 1970). In the present context, localization of the TSP for *hdp*[101] to the mid- to late-pupal stage correlates with the onset of rapid muscle specific gene activity (FALKENTHAL *et al.* 1984) and flight muscle development (CROSSLEY 1978).

Mosaic analysis of *fliA*: Previous studies had shown that mutations in four of the genetically interacting loci directly affect muscle tissue. These include *rsd* (*act88F*) (MAHAFFEY *et al.* 1985), various Mhc mutations (MOGAMI and HOTTA 1981; MOGAMI *et al.* 1986), *up* and *hdp* mutations (DEAK 1977; DEAK *et al.* 1982). To determine the primary tissue affected by mutations in the fifth interacting gene, mosaic analysis was performed to determine the flightless foci (HOTTA and BENZER 1972) for *fliA*[1]. For this, a total of 273 mosaic flies were generated and scored for cuticle and behavioral phenotypes as described in MATERIALS AND METHODS. Of these, 120 displayed mutant behavior. From the facts that less than half of the mosaics (44%) displayed mutant behavior and that three of five bilaterally mosaic flies displayed normal behavior, hopping and flying greater than 9 cm high, the flight defective foci were determined to be submissive, and the data analyzed accordingly

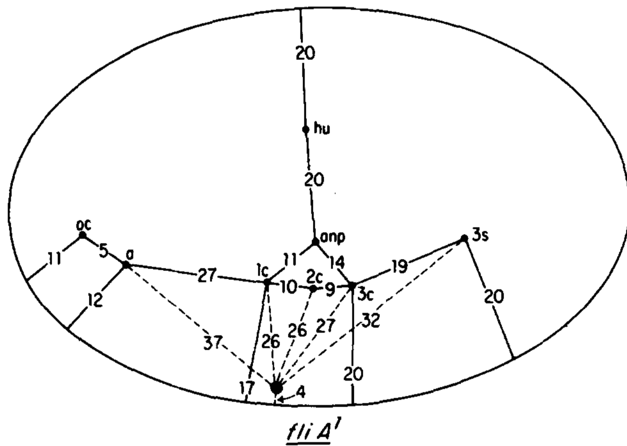

FIGURE 4.—Mosaic analysis of *fliA*. Foci for the flightless phenotype is shown in relation to cuticle foci and ventral midline. Distances indicated are sturts. a, aristae; oc, ocular bristle; anp, anterior notopleural bristle; hu, humerus; 1c, 2c, 3c, first, second and third leg coxae, respectively; 3s, third abdominal sternite.

(HOTTA and BENZER 1972). The results are shown in Figure 4. A bilateral pair of flight defective foci for $fliA^1$ are located midventrally and close to the embryonic midline. Comparisons of the location of these foci with Drosophila embryonic fate maps (POULSON 1965) and with the location of foci known to affect muscle tissue (HOTTA and BENZER 1972; DEAK 1977; KOANA and HOTTA 1978) suggest that these *fliA* foci are in the presumptive mesoderm.

Both present and previous analyses have shown that mutations in all five interacting genes probably affect muscle autonomously. This information together with examples of allele specific interaction, support a biochemical basis involving product interaction to explain the observed intergenic interactions.

DISCUSSION

Successful renaturation of filaments and myofibril subunits from dissociated monomeric units (HARRINGTON and RODGERS 1984; POLLARD 1986) shows that much of the information required for myofibrillar assembly is encoded in the primary structure of the component proteins. *In vivo* studies employing genetic mutations have also shown that the nature of the assembled myofibril is sensitive to the relative quantities of each of its constituents (EPSTEIN, ORTIZ and MACKINNON 1986). Thus, myofibril assembly resembles the assembly process for phage maturation, which has been the subject of intense biochemical and genetic analyses (BLACK and SHOWE 1983; BERGET and KING 1983; WOOD and CROWTHER 1983). By analogy with bacteriophage morphogenesis, myofibrillar development and function should be very sensitive to genetic disruptions that either alter genes coding for the structural components themselves or alter elements that affect the temporal, spatial and quantitative regulation of these genes.

The complex interdependency in the assembly of myofibrillar components and the susceptibility of the assembly process to genetic mutation in Drosophila has been shown by MOGAMI and HOTTA (1981). Rather than affecting the quantity or mobility of a single protein species, different mutations in each of three genes studied, now known to code for the myosin heavy chain (MOGAMI *et al.* 1986), and tropomyosin and flight muscle specific actin (FYRBERG *et al.* 1986), had different effects on the pattern of a set of myofibrillar proteins revealed by two-dimensional gel electrophoresis. The altered patterns displayed both intergenic and interallelic differences. Furthermore, the patterns in the reduction or absence of myofibrillar proteins consistently reflected a hierarchial dependency among the proteins regarding their stable incorporation into the myofibrillar complex.

In view of the above observations, we hypothesized that the myofibrillar complex would be similarly sensitive to genetic mutations in each of the remaining myofibrillar specific components. To test this hypothesis we examined the genetic interactions between muscle defective mutations in thirteen genes in *Drosophila melanogaster*. Our study revealed several types of genetic interactions, including those displaying interallelic and intergenic specificities and those resulting from altered quantitative relationships between gene products. Relevant data, presented fully in Tables 4–6, are summarized in Table 8. The interactions of *fliA* mutations provide a clear example of interallelic and intergenic specificities. Four *fliA* mutations, $fliA^3$, $fliA^4$, *EA82* and $EA82^{HC207}$, interact specifically with the recessive mutation hdp^2 to cause flightlessness (Tables 6 and 8), while a fifth, $fliA^1$, does not. The $fliA^1$ mutation is also unique in that it complements $fliA^4$ (HOMYK, SZIDONYA and SUZUKI 1980). Altogether, these observations are consistent with two explainations. Either $fliA^1$ and $fliA^4$ alter separate functional domains of a single polypeptide, which mutually "compensate" or correct one another in heteropolymers, or the two mutations alter separate, closely linked genes, whose products interact. In either case in comparison to $fliA^4$, the $fliA^1$ mutant product has less (or no) negative interaction with the product of the hdp^2 gene.

Like *fliA*, the *hdp* complementation group displays interallelic complementation and allele specific interactions. Thus, hdp^{102} and hdp^2 show partial complementation (MATERIALS AND METHODS), while neither complements hdp^{101} for flightlessness. While all three mutations interact with int^3, they interact differentially with mutations in other genes, notably Mhc. Thus, hdp^{101} and hdp^{102} interact with *Ifm(2)1* (Tables 5 and 8), while hdp^2 does not. On the other hand, only hdp^2 interacts with a duplication of Mhc^+ and with the Mhc mutations *Bsh*, *Nup*, and *Ifm(2)3* (Tables

TABLE 8

Summary of mutant gene interactions

	Oregon-R													
	Viability	Wing posture	Flight ability	*hdp*^{2}	*hdp*^{3}	*hdp*^{4}	*hdp*^{5}	*hdp*^{101}	*hdp*^{102}	*int*^{3}	*up*^{2}	*up*^{101}	*up*^{X}	*rsd* (*act88F*)
Mhc alleles														
Bsh	+	+	p	l*,wp				wp		l*,wp	wp	wp	wp	
Nup	+	wp	f	l*						l*		l*	l*	
Ifm(2)1	+	+	m					f	f	f		f	f	wp
Ifm(2)3	+	wp	f	l						l		l	l*	
fliA alleles														
fliA^{3}	+	+	+	f										
fliA^{4}	+	+	+	f										
l(1)EA82	+	+	+	f										
l(1)HC207	+	+	+	f										
up alleles														
int^{3}	+	+	+	f,wp	wp	wp	wp	m	m					
up^{2}	+	+	f											wp
up^{101}	+	+	+	f,wp	wp	wp	wp	f	f					wp
up^{x}	+	+	+	f,wp		wp	wp	wp						
hdp alleles														
hdp^{2}	+	+	+											
hdp^{3}	+	+	f											
hdp^{4}	+	+	f											
hdp^{5}	+	+	f											
hdp^{101}	+	+	+											
hdp^{102}	+	+	+											

Matrix summarizing phenotypes of those double mutant combinations which show interactions. Alleles combined are shown in the top row and the left column. Columns 2–4 record the viability, wing posture and flight phenotypes of single heterozygous mutant controls for purposes of comparison. Symbols: "+" = normal, wp = defective wing posture, f = flightless, p = poor flier, m = moderate flier, l = lethal in doubly heterozygous females, l* = combination only lethal in males hemizygous for the *X*-linked mutation. The phenotypes of mutant combinations not showing evidence of interaction have been left blank.

4, 5, 7 and 8). Moreover, *hdp*^{2}, but neither *hdp*^{101} or *hdp*^{102}, interact with *fliA*. As with *fliA*, these data could be explained on the basis that the complementation group defined by *hdp* represents mutations in two closely linked genes whose products interact or a single gene coding for a multifunctional polypeptide. Recent results of molecular genetic analyses make these hypotheses amenable for testing. FALKENTHAL *et al.* (1984) reported a lambda genomic clone, mapping cytologically to 17A, which contains two closely linked, muscle specific coding sequences. This is intriguingly close to the 16F3-8 region shown to contain the *hdp* gene. Thus, it is possible that this gene(s) has been cloned, and a combination of molecular and classical genetic analyses could be used to examine their functional and biophysical interrelationships in the myofibril complex.

Allele-specific differences involving *rsd* deserve mention since this strain carries two mutations affecting flight, one of which is in the act 88F gene (MAHAFFEY *et al.* 1985) coding for flight muscle specific actin. Thus, *rsd* interacts with *up*^{2} and *up*^{101} and the "weakest" Mhc mutation, *Ifm(2)1* to cause abnormal wing posture. No interactions were observed with stronger alleles of Mhc including *Bsh*, *Ifm(2)2*, *Mhc*^{1} or *Stp* to cause abnormal wing posture. In view of the aformentioned observation that *rsd* is cold sensitive, it is notable that its interaction with *up*^{101} is also cold sensitive.

It is also noteworthy that the interactions involving dominant and semidominant flightless mutations in *up* and *hdp* are less severe than those involving recessive mutations in these genes. Thus, none of the dominant or semidominant mutations are lethal in combination with *Mhc* mutations and none interact to affect wing posture or leg mobility as severely as those involving the recessive mutations *hdp*^{2}, *int*^{3}, *up*^{101} or *up*^{x}.

One explanation for the above paradox is that the dominant mutations might drastically reduce the amount or alter the function of the mutant product, and thereby reduce its interaction with other myofibrillar components. Thus, in addition to considering how much mutations reduce their specific gene products function in relation to a specific threshold below which a particular phenotype is expressed (STEPHENS and BRYANT 1986), one must consider how they reduce the function of macromolecular complexes through intermolecular interactions.

The above explanation is negated for *up*^{2} (alias *wupB*) by the finding that *up*^{2} reduces several thoracic myofibrillar specific components in heterozygotes and homozygotes (MOGAMI, NONOMURA and HOTTA 1981), possibly by the interaction of mutant product

with the normal products of other genes. A more plausible explanation is that the present dominant mutations affect fibrillar flight muscle specific exons in differentially spliced genes and, thus, do not affect many of the tubular muscles necessary for viability and other locomotor activities. A precedent for this possibility already exists for the *Mhc* gene (MOGAMI *et al.* 1986). It would be expected that "dominant" mutations in exons used in tubular muscles would drastically reduce viability and seldom be recovered. Thus, predominantly recessive flightless mutations have been found in exons utilized in tubular muscles.

The strict stochiometric relationships among the components of the myofibril suggest that quantitative, as well as qualitative, relationships are important. In the present study, only a limited effort has been made to examine the importance of functional quantitative imbalances to genetic interaction. Nevertheless, this has shown that an extra copy of the Mhc^+ gene interacts "negatively" with recessive flightless mutations in *up* and *hdp* (Table 7). Apparently, the increased imbalance between the ratios of functional products of these genes reduces myofibrillar development and function. In regard to this, it is noteworthy that a subtle change in the relative amounts of myosin A and myosin B gene products in Caenorhabditis alters the distribution of each product along the thick filament in yet undetermined ways (EPSTEIN, ORTIZ and MACKINNON 1986).

The importance of quantitative interactions is also underscored by the discovery that all five genes showing intergenic mutant interactions map to haploinsufficient regions. Previous studies have shown that the *act88F* gene (KARLIK *et al.* 1984) and the *Mhc* gene (MOGAMI *et al.* 1986) are haploinsufficient for flight. Likewise, region 2C, containing *fliA*, region 12A, containing *up* and region 16F, containing *hdp*, have been shown to be haplolethal (STEWART and MERRIAM 1973; TANOUYE, FERRUS and FUJITA 1981; J. MERRIAM, personal communication). Considering that an inversion break at 2C3 in $l(1)EA82^{HC207}$ fails to complement recessive lethal (PERRIMON, ENGSTROM and MAHOWALD 1985) and flightless phenotypes of the *EA82-fliA* complementation group, it might be argued that this gene is not responsible for the haplolethality. It must be noted, however, that the variable lethal period of $EA82^{HC207}$ contrast with the early, sharp lethal period of *EA82* point mutations (PERRIMON, ENGSTROM and MAHOWALD 1985), and the apparent noncomplementation of $EA82^{HC207}$ with relevant mutations could be accounted for by a position effect on a nearby, dosage sensitive gene.

Previous studies had also shown that the 88F region is also haplolethal (LINDSLEY *et al.* 1972). In the light of the present studies, this is probably due to the tropomyosin genes there, since the *act88F* gene is not essential to viability. That no interaction was detected involving *Ifm(3)3*, affecting the tropomyosin gene in 88F (KARLIK and FYRBERG 1986), is possibly accounted for by the fact that the mutation results from an insertion that only reduces the amount of normal gene product. Since it is dominant flightless, interaction would have been recorded only if it caused abnormal wing posture or lethality.

The present study shows that genes coding for interacting myofibrillar proteins reside in three of the four *X*-linked haplolethal regions (Figure 2). It is not yet known whether the fourth haplolethal region, at 7C5-9 (LEFEVRE and JOHNSON 1973), contains such a gene. Muscle defective mutations in two genes, $fliC^2$ in 7B1-8 and *rex* in 7C1-3 (HOMYK *et al.* 1986; our unpublished data; Figure 2) map immediately distal to this region. No interactions were found involving *fliC*. The *rex* mutation has not yet been tested. Efforts are being made to acquire other point lethal mutations (LEFEVRE and WATKINS 1986; LINDSLEY and ZIMM 1986) mapping to this region for relevant testing. If one of these mutations is found to affect myofibril development and to interact with other myofibrillar defective mutations, then the distribution of the four haplolethal regions along the *X* chromosome presents a curious phenomenon. The map positions of the relevant loci *fliA* at 1.0, region 7C at 21, *up* at 41.0 and *hdp* at 59.9, show that they are maximally separated on a recombination map. This arrangement would maximize recombination between naturally occurring variants, which had become established in different populations, at these loci. The possibility that other genes encoding interacting myofibrillar proteins reside in other similarly dispersed haploinsufficient regions and its significance to chromosome organization and evolution are presently under investigation.

Both the extensiveness and specificity among the observed interactions make myofibrillar development and function attractive for further genetic and molecular genetic studies. These studies should identify other members of a large family of myofibrillar genes and aid in the investigation of the biophysical interactions between their products during myofibrillar assembly and function. Indeed, these possibilities are being realized in studies of intergenic interactions involving muscle defective mutations in Caenorhabditis (GREENWALD and HORVITZ 1986; PARK and HORVITZ 1986; LANDEL *et al.* 1984; DIBB *et al.* 1985; WATERSTON *et al.* 1986; EPSTEIN, ORTIZ and MACKINNON 1986) and β-tubulin and other microtubular proteins in Drosophila (FULLER 1986).

Further examination of interactions involving "quantitative" imbalances of myofibrillar gene products could be made using molecular genetic transformation (SPRADLING and RUBIN 1982) to alter the number of normal or mutant gene copies (OKAMOTO *et al.* 1986). Considering that many myofibrillar genes

are differentially expressed, some transformed lines may be useful in genetic screens to detect mutationally altered transregulatory factors. Regarding this, it is noteworthy that fibrillar flight muscle development and function is noticably more sensitive to perturbations in product levels (high threshold) than are tubular muscles (KARLIK and FYRBERG 1986; MOGAMI *et al.* 1986). This, together with the possibility that many myofibrillar genes are differentially expressed and/or spliced in flight muscle (KARLIK and FYRBERG 1986; MOGAMI *et al.* 1986; GEYER and FYRBERG 1986; FALKENTHAL, GRAHAM and WILKINSON 1987), and the knowledge that these muscles are dispensible for viability, make this a model system for genetic studies. These may, then, reveal genes coding for transregulation and RNA processing functions as well as additional structural components themselves.

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